The Sterols of Coconut Oil

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The investigations were first and foremost carried out on sterols from the pitch, which is produced at the distilling of coconut fatty acids ("pitchsterols"), but in addition a preliminary investigation has been made on sterols extracted from crude coconut oil ("crude oil sterols").

For the separation of the sterol mixtures was used fractional crystallization from ethyl ether, ethanol-hexane mixture or propanol, and as a measure for the separation was used the consumption of bromine according to Rosenmund-Kuhnhen.

In the pitch sterols, the presence of α -spinasterol, stigmasterol, β -sitosterol, and fucosterol was proved. No pure compound was isolated from the crude oil sterols, but it contained in addition to the four sterols found in the pitch one sterol with conjugated double bonds in the 5,7-position and one sterol with a 7-double bond.

In search for raw materials for the production of steroide hormones it was found that coconut oil sterols had a comparatively high content of sterols with more than one double bond. As the literature only contained one statement about coconut sterols, namely that they contain stigmasterol¹, an investigation was made, which may not have solved all problems, but which does give a fairly comprehensive account of the composition of this complicated mixture.

The coconut sterols were obtained from the fatty pitch which results from the distillation of the soap-stock fatty acids from the refining of coconut oil. These sterols will be referred to as "pitch sterols". Besides, a preliminary investigation has been made on sterols from crude coconut oil ("crude oil sterols").

For an initial characterization of the two kinds of sterols, the following determinations were made:

1. By saponification of the acetate, the average molecular weight of the pitch sterols was determined as 414 (29 carbon atoms).
2. On quantitative hydrogenation of the pitch sterols, 1.31 moles H_2 per mole sterol were consumed, which means that there is 31 % sterol with two hydrogenatable double bonds. In the same way the crude oil sterols were found to contain 36 % sterols with two hydrogenatable double bonds.

3. On bromination according to Rosenmund-Kuhnhehn ², 1.53 moles bromine per mole pitch sterol and 1.84 moles bromine per mole crude oil sterol were consumed. This means that substitution takes place at the brominations, and that sterols with non-hydrogenatable double bonds may occur.

4. Idler and Baumann's modified Lieberman-Burchard reaction ³ shows that the pitch sterols contain 3 %, and the crude oil sterols 10 % sterol with a double bond at carbon atom No. 7.

5. The pitch sterols have only a negligible absorption in the ultraviolet region down to 2 500 Å, so that sterols with conjugated double bonds cannot occur. The crude oil sterols, on the other hand, have their absorption maxima at 2 930, 2 820, and 2 715 Å, which is characteristic of sterols with conjugated double bonds in the 5,7 position ⁴. The intensities of the maxima indicate 4.5 % sterol of this kind.

6. Addition of bromine to the acetate of the pitch sterols according to Windaus and Haut ⁵ produced a precipitate corresponding to 18—20 % stigmasteryl acetate in the pitch sterols. This precipitate, however, was a mixture, and only after a fractional crystallization from ethanol, pure tetrabromostigmasteryl acetate was obtained in a quantity corresponding to 4—8 % stigmasteryl acetate in the pitch sterol. Some derivatives of stigmasteryl acetate have been prepared, and their melting points and specific rotations are compared with values from the literature (Table 1).

THE PITCH STEROLS

The pitch sterols were further investigated by fractional crystallization. A number of solvents was examined for their fractionating power, the change in the consumption of bromine after one re-crystallization being used as the criterion. Ethyl ether proved to be the best, but considerable fractionation was also obtained from a mixture consisting of equal portions of 96 % ethanol and hexane, and from *n*-propanol.

The fractional crystallization was performed in the usual way ⁶. So much solvent was used that about half the quantity of substance was crystallized off each time. First ethyl ether was applied, and after 10 series of crystallizations, the least soluble component, fraction A, was found to consume 3.10 moles bromine per mole sterol, whereas the most soluble component, fraction B, consumed 1.25 moles bromine. Further crystallization from ether brought no alteration in these values.

Fraction A could not be separated into its constituents by crystallization as sterol or acetate. From the benzoate pure stigmasteryl benzoate was isolated by crystallization from a mixture of chloroform and ethanol, while other components could not be isolated.

Eventually the separation was successful by fractional crystallization of the ketone (Oppenauer-oxidation ⁷) from ethanol, and pure α -spinastadienone was isolated as the less soluble and stigmastadienone as the more soluble component.

Fraction A, then, is a mixture of α -spinasterol and stigmasteryl acetate, and a quantitative hydrogenation shows that it consists of 70 % stigmasteryl acetate and 30 % α -spinasterol, the 7-double bond not being hydrogenated. The optical rotation of sterol, acetate, and benzoate, is in accordance with this (Table 1).

Table 1. Melting points and optical rotations of sterols and their derivatives.

Ref. No.	Stigmasterol				α -Spinasterol				Fraction A			β -Sitosterol				γ -Sitosterol	
	m.p.		$[\alpha]_D$		m.p.		$[\alpha]_D$		m.p.		$[\alpha]_D$	m.p.		$[\alpha]_D$		m.p. $[\alpha]_D$	
	Exp	7,15	Exp	7,15	Exp	16,17	Exp	16,17	Exp	Exp	*	Exp	7,18	Exp	7,18	19,20	19,20
Sterol	169	168	-51	-51	—	169	—	-2.7	163	-35	-36	138	136	-36	-37	147	-43
Acetate	147	143	-55	-55	—	180	—	-6.4	143	-38	-40	126	125	-40	-41	143	-48
Benzoate	162	164	-25	-26	—	196	—	+2.1	156	-18	-18	149	146	-15	-14	152	-19
Ketone Stanyl- acetate	124	126	+60	+62	176	176	22	20	—	—	—	—	—	—	—	—	—
	131	131	+14	+15	—	—	—	—	—	—	—	133	131	+12	+15	143	+9—
																—44	10

* Calculated for 30 % α -spinasterol and 70 % stigmasterol.

Fraction B consumed 1.25 moles bromine per mole sterol, and as mentioned above, fractional crystallization from ethyl ether gave no further separation. But by a great number of fractional crystallizations from a mixture of ethanol and hexane (1:1), the following four main fractions were obtained: B 1 consuming 1.50, B 2 1.17, B 3 1.65, and B 4 1.50 moles bromine per mole sterol.

In fraction B 1 stigmasterol and α -spinasterol have again been concentrated.

Fraction B 2 was exposed to fractional crystallization from *n*-propanol, and it was possible to reduce the consumption of bromine to 1.08 moles per mole, which figure was also found on quantitative hydrogenation. Although, then, the substance is not entirely pure, melting points and optical rotations of sterol, acetate, and benzoate show that it is β -sitosterol (Table 1).

Fraction B 3 could not be further separated. Attempts were made with several other solvents and with fractional crystallization of ketone and benzoate; but in no case could a pure substance be isolated.

As mentioned above, the fraction B 3 consumes 1.65 moles bromine per mole sterol, and the same value is found on a quantitative hydrogenation, so that the mixture must consist of 35 % of a sterol with one double bond and 65 % of a sterol with two double bonds.

On ozonizing according to Mac Phillamy⁸ acetaldehyde was isolated as carboxyphenylhydrazone and as 2,4-dinitrophenylhydrazone, the latter in a yield corresponding to 32 % of the available quantity of sterol with two double bonds.

On ozonizing the acetate according to Idler and Fagerlund⁹ acetaldehyde was again obtained as 2,4-dinitrophenylhydrazone, while the non-volatile portion was chromatographed on a mixture of celite and silicagel, as described by Idler and Fagerlund. Unreacted sterol was eluted with benzene, after which a ketone was eluted with a mixture of benzene and chloroform (4:1 by volume).

Melting point and optical rotation of this ketone, as well as the melting point of the 2,4-dinitrophenylhydrazone showed that the ketone was 24-ketocholesterylacetate, whereby the easily soluble component with 2 double bonds has been identified as fucosterol.

Fraction B 4 is slightly yellow, indicating that it contains decomposition products of the sterols. In the ultraviolet absorption curve of the fraction there is an obvious maximum at 2 400 Å, whereas all the other fractions here have only a faint shoulder on the absorption curve rising steeply towards the absorption maximum of the isolated double bonds at 2 050 Å.

The maximum at 2 400 Å is no doubt caused by a conjugated system of $C=C$ and $C=O$ -double bonds, a ketone group at the allylic 7-carbon atom constituting such a system with the C-5-double bond. This theory is in accordance with Bergström and Wintersteiner¹⁰ who have shown that by oxidation of a colloid solution of cholesterol with oxygene, the 7-keto compound is actually produced, this compound having its absorption maximum at 2 400 Å.

Fraction B 4 consumes less bromine than fraction B 3, and this is due to the fact that the conjugated system is more stable than the isolated double bond. Thus cholestenone, which has a conjugated system analogous with the 7-ketosterols, consumes only approximately 25 % of the theoretical quantity of bromine at the Rosenmund-Kuhnhehn-bromination.

As will be mentioned later the more soluble fractions of the crude oil sterols contain sterols with a double bond at C-7, but no evidence of such sterols was found in fraction B 4 from the pitch sterols.

Consequently, fraction B 4 is supposed to consist of the same sterols as fraction B 3, but contaminated by 7-ketosterols formed during the many crystallizations.

Hence, the structure of the pitch sterols must be as shown in Table 2, the percentages being rather uncertain.

THE CRUDE OIL STEROLS

The attempt at isolating pure substances from the crude oil sterols was unsuccessful, but it is, however, possible to indicate their composition with some accuracy.

On fractional crystallization from ethanol-hexane mixture (1:1) the sterol with conjugated double bonds was concentrated in the least soluble fractions, together with stigmasterol and possibly α -spinasterol. A quantitative hydrogenation showed that the conjugated sterol has 2 hydrogenatable double bonds. Hence, there is some reason to presume that it is $\Delta^{5,7,22}$ -stigmastatrienol.

At the fractional crystallization an additional series of fractions of medium solubility was obtained, probably consisting of fucosterol and β -sitosterol, and, finally, an easily soluble fraction, which shows strong substitution at bromination. Presumably it contains a Δ^7 -stenol, analogous with Fieser's lathosterol¹¹, which is also concentrated in the more soluble fractions.

Hence, the composition of the crude oil sterols can be as indicated in Table 2.

When the composition of the two kinds of sterols is compared, it is seen that the sterol with the conjugated double bonds has been totally destroyed

All the sterols from coconut oil are derivatives of stigmasterol.

Table 2. The composition of the sterols from coconut oil.

	Double bonds	Pitch Sterols, %	Crude Oil Sterols, %
Stigmastatrienol	5, 7, 22	0	4.5
Stigmasterol	5, 22	6-10	} 31.5
Fucosterol	5, 24 (28)	20-25	
α -Spinasterol	7, 22	3	} 6.0
Stenol	7	0	
β -Sitosterol	5	66	58.0

at the high temperature during the distillation of the fatty acids, whereas the quantity of sterols with an isolated double bond at C-7 has been considerably reduced. On the other hand, the ratio of the quantity of β -sitosterol to the total quantities of stigmasterol and fucosterol has not been substantially changed.

EXPERIMENTAL

The melting points were determined on Kofler Heizbank and optical rotations in 1 dm macro tubes (about 0.2 g in 10.0 ml chloroform). U.V.spectra were measured on a Beckman model DU instrument.

To obtain the pitch sterols, the pitch, which contained 15-30 % unsaponifiable matter, was saponified with alcoholic KOH and extracted with hexane. After the distilling of the hexane a viscous, dark brown substance containing 50-70 % sterol was obtained. By re-crystallization from a mixture consisting of equal portions of hexane and ethanol, the pitch sterols were obtained as a yellowish product, and it was difficult to obtain perfectly white crystals. Accordingly, the yield was only 50-60 % of the sterols in the pitch.

From crude coconut oil 0.17 % unsaponifiable matter was obtained, and from this again 30 % sterol.

Bromination according to Rosenmund-Kuhnnehn was carried out according to *Handbuch der Lebensmittelchemie*². Surplus of bromide (15-25 %) was titrated iodometrically. The transformation into acetate, recommended by B.I.O.S.¹² was found unnecessary.

Benzoates and acetates were prepared by the usual methods. The yields were 90-95 % of theory. Derivatives of fraction A were only re-crystallized once in order to avoid

fractionation, while pure substances were re-crystallized until the melting points remained constant.

Quantitative hydrogenations were carried out in an apparatus which was a modification of the apparatus of Prater and Haagen-Smit¹³, and of Savacool and Ulliyot¹⁴.

Derivatives of fucosterol: 2,4-dinitrophenylhydrazone of acetaldehyde m.p. 162–64° (lit. value²¹ 163.5–64.5°); *p*-carboxyphenylhydrazone of acetaldehyde m.p. 209–10° (210–11°). Molecular weight found²² 178 and 186 (theoretical 178). 24-Ketocholesterylacetate m.p. 126.5°, $[\alpha]_D$ –42° (lit. value⁹ 126–27° and –43°). 2,4-Dinitrophenylhydrazone of 24-ketocholesterylacetate m.p. 169° (lit. value⁹ 170°).

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